PATENT COOPERATION TREATY

From the INTERNATIONAL BUREAU

PCT

NOTIFICATION OF ELECTION

(PCT Rule 61.2)

To:

Assistant Commissioner for Patents
United States Patent and Trademark Office
Box PCT
Washington, D.C.20231
ETATS-UNIS D'AMERIQUE

in its capacity as elected Office

Date of mailing (day/month/year) 07 August 2000 (07.08.00)	
International application No. PCT/GB99/04045	**Applicant's or agent's file reference** KEG/40325
International filing date (day/month/year) 02 December 1999 (02.12.99)	**Priority date** (day/month/year) 03 December 1998 (03.12.98)
Applicant Qi, Steve et al	

1. The designated Office is hereby notified of its election made:

[X] *in the demand filed with the International Preliminary Examining Authority on:*

27 June 2000 (27.06.00)

[ ] in a notice effecting later election filed with the International Bureau on:

2. The election [X] was

[ ] was not

made before the expiration of 19 months from the priority date or, where Rule 32 applies, within the time limit under Rule 32.2(b).

The International Bureau of WIPO **34, chemin des Colombettes** **1211 Geneva 20, Switzerland**	Authorized officer Pascal Piriou
Facsimile No.: (41-22) 740.14.35	Telephone No.: (41-22) 338.83.38

Copy for the designated Office (DO/US) PCT/GB99/04045

PATENT COOPERATION TREATY

From the INTERNATIONAL BUREAU

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NOTIFICATION OF THE RECORDING OF A CHANGE

(PCT Rule 92bis.1 and Administrative Instructions, Section 422)

To:

GEERING, Keith, Edwin
Reddie & Grose
16 Theobalds Road
London WC1X 8PL
ROYAUME-UNI

Date of mailing (day/month/year)
27 April 2000 (27.04.00)

Applicant's or agent's file reference
KEG/40325

IMPORTANT NOTIFICATION

International application No.
PCT/GB99/04045

International filing date (day/month/year)
02 December 1999 (02.12.99)

1. The following indications appeared on record concerning:

[X] the applicant [ ] the inventor [ ] the agent [ ] the common representative

Name and Address
FERRING BV
Marsstraat 9
P.O. Box 3129
NL-2130 KC Hoofddorp
Netherlands

State of Nationality: NL
State of Residence: NL
Telephone No.:
Facsimile No.:
Teleprinter No.:

2. The International Bureau hereby notifies the applicant that the following change has been recorded concerning:

[ ] the person [ ] the name [X] the address [ ] the nationality [ ] the residence

Name and Address
FERRING BV
Polaris Avenue 144
NL-2132 JX Hoofddorp
Netherlands

State of Nationality: NL
State of Residence: NL
Telephone No.:
Facsimile No.:
Teleprinter No.:

3. Further observations, if necessary:

4. A copy of this notification has been sent to:

[X] the receiving Office
[X] the International Searching Authority
[ ] the International Preliminary Examining Authority
[X] the designated Offices concerned
[ ] the elected Offices concerned
[ ] other:

The International Bureau of WIPO
34, chemin des Colombettes
1211 Geneva 20, Switzerland
Facsimile No.: (41-22) 740.14.35

Authorized officer
I. Britel
Telephone No.: (41-22) 338.83.38

ATENT COOPERATION TREATY

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INTERNATIONAL SEARCH REPORT

(PCT Article 18 and Rules 43 and 44)

Applicant's or agent's file reference	**FOR FURTHER ACTION** see Notification of Transmittal of International Search Report (Form PCT/ISA/220) as well as, where applicable, item 5 below.	
KEG/40325		
International application No.	International filing date *(day/month/year)*	(Earliest) Priority Date *(day/month/year)*
PCT/GB 99/04045	02/12/1999	03/12/1998
Applicant		
FERRING BV et al.		

This International Search Report has been prepared by this International Searching Authority and is transmitted to the applicant according to Article 18. A copy is being transmitted to the International Bureau.

This International Search Report consists of a total of 3 sheets.

[X] It is also accompanied by a copy of each prior art document cited in this report.

1. **Basis of the report**

   a. With regard to the **language**, the international search was carried out on the basis of the international application in the language in which it was filed, unless otherwise indicated under this item.

   [ ] the international search was carried out on the basis of a translation of the international application furnished to this Authority (Rule 23.1(b)).

   b. With regard to any **nucleotide and/or amino acid sequence** disclosed in the international application, the international search was carried out on the basis of the sequence listing :

   [X] contained in the international application in written form.

   [X] filed together with the international application in computer readable form.

   [ ] furnished subsequently to this Authority in written form.

   [ ] furnished subsequently to this Authority in computer readble form.

   [X] the statement that the subsequently furnished written sequence listing does not go beyond the disclosure in the international application as filed has been furnished.

   [X] the statement that the information recorded in computer readable form is identical to the written sequence listing has been furnished

2. [ ] **Certain claims were found unsearchable** (See Box I).

3. [ ] **Unity of invention is lacking** (see Box II).

4. With regard to the **title**,

   [ ] the text is approved as submitted by the applicant.

   [X] the text has been established by this Authority to read as follows:

   CONTROLLED RELEASE FORMULATION COMPRISING GNRH-II

5. With regard to the **abstract**,

   [X] the text is approved as submitted by the applicant.

   [ ] the text has been established, according to Rule 38.2(b), by this Authority as it appears in Box III. The applicant may, within one month from the date of mailing of this international search report, submit comments to this Authority.

6. Th figure of th **drawings** to be publish d with the abstract is Figure No. _____

   [ ] as suggested by the applicant.

   [ ] because the applicant failed to suggest a figure.

   [ ] because this figure better charact rizes th invention.

   [X] Non of the figures.

INTERNATIONAL SEARCH REPORT

International Application No
PCT/GB 99/04045

A. CLASSIFICATION OF SUBJECT MATTER
IPC 7 A61K38/09 A61K47/34

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC 7 A61K

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	WO 97 47743 A (ZYMOGENETICS INC ;UNIV CAPE TOWN (ZA)) 18 December 1997 (1997-12-18) page 24, line 3 - line 29	1-5
Y	SHARP P.J. ET AL: "Effect of luteinising hormone releasing hormone and its analogues on plasma luteinising hormone concentrations in incubating bantam hens" BRITISH POULTRY SCIENCE, vol. 27, no. 1, 1986, pages 129-136, XP000885654 abstract	1-5
Y	US 4 835 139 A (TICE THOMAS R ET AL) 30 May 1989 (1989-05-30) claims	1-5

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[X] Further documents are listed in the continuation of box C.

[X] Patent family members are listed in annex.

° Special categories of cited documents :

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier document but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
26 April 2000	04/05/2000

Name and mailing address of the ISA	Authorized officer
European Patent Office, P.B. 5818 Patentlaan 2 NL - 2280 HV Rijswijk Tel. (+31-70) 340-2040, Tx. 31 651 epo nl, Fax: (+31-70) 340-3016	Seegert, K

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Form PCT/ISA/210 (second sheet) (July 1992)

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication,where appropriate, of the relevant passages	Relevant to claim No.
Y	ASANO M ET AL: "IN VIVO CHARACTERISTICS OF LOW MOLECULAR WEIGHT COPOLY(L-LACTIC ACID/GLYCOLIC ACID) FORMULATIONS WITH CONTROLLED RELEASE OF LUTEINIZING HORMONE-RELEASING HORMONE AGONIST" JOURNAL OF CONTROLLED RELEASE,NL,ELSEVIER SCIENCE PUBLISHERS B.V. AMSTERDAM, vol. 9, no. 2, 1 July 1989 (1989-07-01), pages 111-122, XP000611562 ISSN: 0168-3659 abstract	1-5
A	EP 0 158 987 A (HOECHST AG) 23 October 1985 (1985-10-23) claims	1-5
A	WHITE R B ET AL: "Second gene for gonadotropin-releasing hormone in humans." PROCEEDINGS OF THE NATIONAL ACADEMY OF SCIENCES OF THE UNITED STATES OF AMERICA, (1998 JAN 6) 95 (1) 305-9. , XP000882990 cited in the application abstract	1-5

1

INTERNATIONAL SEARCH REPORT

[redacted]nation on patent family members

[redacted]national Application No
PCT/GB 99/04045

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
WO 9747743 A	18-12-1997	AU 3388597 A	07-01-1998
US 4835139 A	30-05-1989	CH 661206 A	15-07-1987
EP 0158987 A	23-10-1985	DE 3414595 A	31-10-1985
		AT 49980 T	15-02-1990
		AU 575151 B	21-07-1988
		AU 4136585 A	24-10-1985
		DK 174085 A,B,	19-10-1985
		GR 850940 A	25-11-1985
		JP 60233017 A	19-11-1985
		US 4788178 A	29-11-1988
		ZA 8502855 A	27-11-1985

PATENT COOPERATION TREATY

PCT

INTERNATIONAL PRELIMINARY EXAMINATION REPORT

(PCT Article 36 and Rule 70)

Applicant's or agent's file reference	FOR FURTHER ACTION	See Notification of Transmittal of International Preliminary Examination Report (Form PCT/IPEA/416)
KEG/40325		
International application No.	International filing date *(day/month/year)*	Priority date *(day/month/year)*
PCT/GB99/04045	02/12/1999	03/12/1998
International Patent Classification (IPC) or national classification and IPC A61K38/09		
Applicant FERRING BV et al.		

1. This international preliminary examination report has been prepared by this International Preliminary Examining Authority and is transmitted to the applicant according to Article 36.

2. This REPORT consists of a total of 7 sheets, including this cover sheet.

   ☒ This report is also accompanied by ANNEXES, i.e. sheets of the description, claims and/or drawings which have been amended and are the basis for this report and/or sheets containing rectifications made before this Authority (see Rule 70.16 and Section 607 of the Administrative Instructions under the PCT).

   These annexes consist of a total of 2 sheets.

3. This report contains indications relating to the following items:

   - I ☒ Basis of the report
   - II ☐ Priority
   - III ☒ Non-establishment of opinion with regard to novelty, inventive step and industrial applicability
   - IV ☐ Lack of unity of invention
   - V ☒ Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations suporting such statement
   - VI ☐ Certain documents cited
   - VII ☐ Certain defects in the international application
   - VIII ☒ Certain observations on the international application

Date of submission of the demand	Date of completion of this report
27/06/2000	15.02.2001
Name and mailing address of the international preliminary examining authority: European Patent Office D-80298 Munich Tel. +49 89 2399 - 0 Tx: 523656 epmu d Fax: +49 89 2399 - 4465	Authorized officer Seegert, K Telephone No. +49 89 2399 8409

Form PCT/IPEA/409 (cover sheet) (January 1994)

INTERNATIONAL PRELIMINARY EXAMINATION REPORT

International application No. PCT/GB99/04045

I. Basis of the report

1. This report has been drawn on the basis of *(substitute sheets which have been furnished to the receiving Office in response to an invitation under Article 14 are referred to in this report as "originally filed" and are not annexed to the report since they do not contain amendments (Rules 70.16 and 70.17).)*:
Description, pages:

1-14	as originally filed	

Claims, No.:

1-6	as originally filed	
7-13	with telefax of	27/06/2000

Drawings, sheets:

1/1	as originally filed	

2. With regard to the **language**, all the elements marked above were available or furnished to this Authority in the language in which the international application was filed, unless otherwise indicated under this item.

   These elements were available or furnished to this Authority in the following language: , which is:

   - ☐ the language of a translation furnished for the purposes of the international search (under Rule 23.1(b)).
   - ☐ the language of publication of the international application (under Rule 48.3(b)).
   - ☐ the language of a translation furnished for the purposes of international preliminary examination (under Rule 55.2 and/or 55.3).

3. With regard to any **nucleotide and/or amino acid sequence** disclosed in the international application, the international preliminary examination was carried out on the basis of the sequence listing:

   - ☐ contained in the international application in written form.
   - ☐ filed together with the international application in computer readable form.
   - ☐ furnished subsequently to this Authority in written form.
   - ☐ furnished subsequently to this Authority in computer readable form.
   - ☐ The statement that the subsequently furnished written sequence listing does not go beyond the disclosure in the international application as filed has been furnished.
   - ☐ The statement that the information recorded in computer readable form is identical to the written sequence listing has been furnished.

4. The amendments have resulted in the cancellation of:

- ☐ the description, pages:
- ☐ the claims, Nos.:
- ☐ the drawings, sheets:

5. ☐ This report has been established as if (some of) the amendments had not been made, since they have been considered to go beyond the disclosure as filed (Rule 70.2(c)):

   (Any replacement sheet containing such amendments must be referred to under item 1 and annexed to this report.)

6. Additional observations, if necessary:

III. Non-establishment of opinion with regard to novelty, inventive step and industrial applicability

1. The questions whether the claimed invention appears to be novel, to involve an inventive step (to be non-obvious), or to be industrially applicable have not been examined in respect of:

   ☐ the entire international application.

   ☒ claims Nos. 6,12,13 with respect to IA.

because:

   ☒ the said international application, or the said claims Nos. 6,12,13 relate to the following subject matter which does not require an international preliminary examination (*specify*):
   see separate sheet

   ☐ the description, claims or drawings (*indicate particular elements below*) or said claims Nos. are so unclear that no meaningful opinion could be formed (*specify*):

   ☐ the claims, or said claims Nos. are so inadequately supported by the description that no meaningful opinion could be formed.

   ☐ no international search report has been established for the said claims Nos. .

2. A meaningful international preliminary examination report cannot be carried out due to the failure of the nucleotide and/or amino acid sequence listing to comply with the standard provided for in Annex C of the Administrative Instructions:

   ☐ the written form has not been furnished or does not comply with the standard.

   ☐ the computer readable form has not been furnished or does not comply with the standard.

V. Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement

1. Statement

Novelty (N)	Yes:	Claims	1-13
	No:	Claims	
Inventive step (IS)	Yes:	Claims	1-13
	No:	Claims	
Industrial applicability (IA)	Yes:	Claims	1-5,7-11
	No:	Claims	

2. Citations and explanations
see separate sheet

VIII. Certain observations on the international application

The following observations on the clarity of the claims, description, and drawings or on the question whether the claims are fully supported by the description, are made:
see separate sheet

Section III

1. Claims 6, 12, 13 relate to subject-matter considered by this Authority to be covered by the provisions of Rule 67.1(iv) PCT. Consequently, no opinion will be formulated with respect to the industrial applicability of the subject-matter of these claims (Article 34(4)(a)(i) PCT).

Section V

1. Reference is made to the following documents:

   D1: WO-A-9747743
   D2: British Poultry Science (1986), 27(1), 129-136
   D3: US-A-4835139
   D4: Journal Of Controlled Release, NL, Elsevier Science Publishers B.v. Amsterdam (01-07-1989), 9(2), 111-122
   D5: EP-A-0 158 987 (HOECHST AG) 23 October 1985 (1985-10-23)

   If not indicated otherwise, the relevant passages are those mentioned in the International Search Report.

2. The subject-matter of the present application relates to pharmaceutical compositions for sustained release comprising GnRH-II and a pharmaceutically acceptable biodegradable polymer, in particular a copolymer of lactic and glycolic acid and to their use in therapy.

3. The subject-matter of claims 1 - 13 meets the novelty requirements of Article 33 (2) PCT, since the prior art does not disclose the sustained release formulations of the present application.

3. With respect to inventive step the following comments are made:

Document D1 discloses the receptor for GnRH-II. It also suggests the potential use of GnRH-II in therapy based on the known therapeutic effects of GnRH. However the document does not provide any results of the alleged therapeutic use.

Document D2 discloses the pharmacological effects of GnRH-II on LH release without referring to any therapeutic application. It appears also from D2 that there is a difference in the pharmacological activity of GnRH and GnRH-II.

Documents D3 - D5 relate to formulations and therapeutic use of GnRH and analogs.

In view of the documents cited above it appears that there is no clear pointer in the prior art which would have guided the skilled person to therapeutically active formulations of GnRH-II and to their use in therapy as claimed. This applies in particular since the only document referring to a potential use of GnRH-II (i.e. D1) is quite speculative on this point and mainly based on the alleged similarity between GnRH-II and GnRH. However, this allegation does not seem to be supported by the teaching of D2.

Therefore, it appears that the subject-matter as claimed in claims 1 - 13 is not obvious to the skilled person and thus involves an inventive step. Consequently, the requirements of Article 33 (3) PCT are met.

4. For the assessment of the present claim 6 - 13 on the question whether it is industrially applicable, no unified criteria exist in the PCT Contracting States. The patentability can also be dependent upon the formulation of the claims. The EPO, for example, does not recognize as industrially applicable the subject-matter of claims to the use of a compound in medical treatment, but may allow, however, claims to a known compound for first use in medical treatment and the use of such a compound for the manufacture of a medicament for a new medical treatment.

Section VIII

The subject-matter of claim 6 lacks clarity within the meaning of Article 6 PCT since the claim does not specify the therapeutic application.

PCT WORLD INTELLECTUAL PROPERTY ORGANIZATION
International Bureau

INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(51) International Patent Classification 7 : A61K 38/09, 47/34

A1

(11) International Publication Number: **WO 00/32218**

(43) International Publication Date: 8 June 2000 (08.06.00)

(21) International Application Number: PCT/GB99/04045

(22) International Filing Date: 2 December 1999 (02.12.99)

(30) Priority Data:
9826662.0 3 December 1998 (03.12.98) GB

(71) Applicant *(for all designated States except US)*: FERRING BV [NL/NL]; Polaris Avenue 144, NL–2132 JX Hoofddorp (NL).

(72) Inventors; and

(75) Inventors/Applicants *(for US only)*: QI, Steve [GB/GB]; 69 Downscroft Gardens, Hedge End, Southampton SO30 4RS (GB). AKINSANYA, Karen [GB/GB]; 38 Macnaghten Road, Bitterne Park, Southampton SO18 1GJ (GB). HAYWARD, Amanda [GB/GB]; 15 Chesterton Hall Crescent, Cambridge CB4 1AW (GB).

(74) Agent: GEERING, Keith, Edwin; Reddie & Grose, 16 Theobalds Road, London WC1X 8PL (GB).

(81) Designated States: AE, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, CA, CH, CN, CR, CU, CZ, DE, DK, DM, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, TZ, UA, UG, US, UZ, VN, YU, ZA, ZW, ARIPO patent (GH, GM, KE, LS, MW, SD, SL, SZ, TZ, UG, ZW), Eurasian patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European patent (AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, GW, ML, MR, NE, SN, TD, TG).

Published
With international search report.
Before the expiration of the time limit for amending the claims and to be republished in the event of the receipt of amendments.

(54) Title: CONTROLLED RELEASE FORMULATION COMPRISING GNRH–II

(57) Abstract

A pharmaceutical formulation for the controlled release of a therapeutic peptide or a salt thereof, which peptide has the sequence pyroGlu–His–Trp–Ser–Xaa^1–Gly–Xaa^2–Xaa^3–Pro–Gly–NH_2 wherein Xaa^1 is His or Tyr, Xaa^2 is Trp or Leu, and Xaa^3 is Tyr or Arg, provided that when Xaa^1 is Tyr and Xaa^2 is Leu, then Xaa^3 is not Arg, and which formulation further comprises a pharmaceutically acceptable biodegradable polymer. The formulation can be used for treating bone and prostate disorders.

FOR THE PURPOSES OF INFORMATION ONLY

Codes used to identify States party to the PCT on the front pages of pamphlets publishing international applications under the PCT.

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CONTROLLED RELEASE FORMULATION COMPRISING GNRH-II

FIELD OF INVENTION

The present invention relates to a pharmaceutical preparation that releases a therapeutic agent over an extended period.

BACKGROUND TO THE INVENTION

Studies on the physiology of the hypothalamic-pituitary-gonadal axis have resulted in the recognition of gonadotropin releasing hormone (GnRH. otherwise known as luteinizing hormone releasing hormone, LHRH) as a key regulatory hormone. GnRH is released by the hypothalamus and acts on the pituitary to stimulate the release of luteinizing hormone (LH) and follicle-stimulating hormone (FSH). More recently, a peptide with homology to GnRH has been identified (White *et al.*, Proc. Natl. Acad. Sci. USA 95 305-309, 1998). This peptide has been called GnRH-II. The sequences of the two peptides are compared below.

GnRH	pyroGlu-His-Trp-Ser-**Tyr**-Gly-**Leu-Arg**-Pro-Gly-NH_2	(SEQ I.D. No.5)
GnRH-II	pyroGlu-His-Trp-Ser-**His**-Gly-**Trp-Tyr**-Pro-Gly-NH_2	(SEQ I.D. No.6)

The name "GnRH-II" is, to some extent, misleading. The new peptide is a separate gene product, and is clearly distinguishable from GnRH in its tissue distribution. It seems unlikely that GnRH-II acts as an endogenous releaser of LH and FSH. Since no clear evidence for a physiological role for GnRH-II has been presented, no attentionhas been paid to the practical aspects of using this peptide as a therapeutic agent.

SUMMARY OF THE INVENTION

We have now found that GnRH-II has an important role in the function of a number of organs. For example, it influences osteogenesis and it modulates the proliferation of prostatic epithelial cells. Accordingly, we have considered the means by which this agent and its analogues might usefully be delivered in a clinical situation. and it is an object of the present invention to provide suitable formulations for achieving this purpose. The formulations according to the present invention rely on the use of a

biodegradable polymer to hold the peptide in a depot, from which it is released into the systemic circulation at a controlled rate. These formulations comprise two key elements, the biologically active peptide and the biodegradable polymer. The biologically active peptide is a decapeptide according to the sequence

pyroGlu-His-Trp-Ser-**Xaa**^{1}-Gly-**Xaa**^{2}-**Xaa**^{3}-Pro-Gly-NH_2 (SEQ I.D. No.7)

wherein **Xaa**^{1} is His or Tyr,
Xaa^2 is Trp or Leu, and
Xaa^3 is Tyr or Arg,

provided that when **Xaa**^{1} is Tyr and **Xaa**^{2} is Leu, then **Xaa**^{3} is not Arg. The polymer is any pharmaceutically acceptable biodegradable polymer, and preferably a co-polymer of glycolic and lactic acids. The invention further comprises the use of the formulations for the treament of human pathologies.

DESCRIPTION OF THE FIGURE

Figure 1 shows the effect of increasing doses of GnRH-II on serum calcium concentrations in ovariectomised rats.

DESCRIPTION OF THE INVENTION

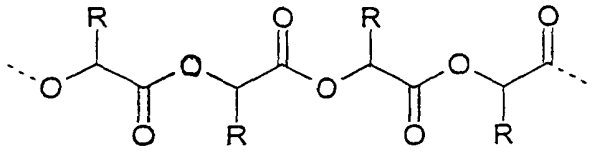
As used herein, abbreviations referring to amino acids have their conventional meanings and indicate the natural L-isomer (except for the achiral amino acid glycine).

In a first aspect, the invention as disclosed herein comprises a pharmaceutical formulation that releases a therapeutic peptide at a controlled rate and for an extended period of time (i.e. for a period of at least one day, preferably several days, and more preferably at least one week), particularly for the treatment of diseases of the bone and prostate. The therapeutic peptide is a decapeptide according to the sequence

pyroGlu-His-Trp-Ser-**Xaa**^{1}-Gly-**Xaa**^{2}-**Xaa**^{3}-Pro-Gly-NH_2 (7)

wherein **Xaa**[1] is either His or Tyr, **Xaa**[2] is either Trp or Leu, and **Xaa**[3] is either Tyr or Arg, provided that when **Xaa**[1] is Tyr and **Xaa**[2] is Leu, then **Xaa**[3] is not Arg. Preferably, **Xaa**[1] is His, **Xaa**[2] is Trp, and **Xaa**[3] is Tyr. It will be recognised that such a peptide can form salts with acids (for example, acetic acid, trifluoroacetic acid, benzoic acid, hydrochloric acid, phosphoric acid and the like). To the extent that such salts are formed with pharmaceutically acceptable acids, they are included within the scope of the invention.

A second essential component of the formulation is a biodegradable, pharmaeutically acceptable polymer. Such polymers are known in the art. They can either be homopolymers (i.e. polymers of a single monomer) or copolymers (i.e. formed from two or more different monomers). Suitable monomers include amino and hydroxy dervatives of carboxylic acids. In a preferred embodiment of the present invention, the polymer is composed of hydroxyacyl monomeric units, and more preferably of α-hydroxyacyl units. Most preferably, the polymer is a poly(glycolic acid), a poly(lactic acid) or a copolymer of glycolic and lactic acids. Such a polymer has the following chemical structure.



where R is hydrogen in poly(glycolic acid), methyl in poly(lactic acid), and randomly hydrogen or methyl in the copolymer.

Two complementary methods for making the formulation of the present invention can be distinguished. The peptide can either be incorporated into a matrix of the polymer, or, more preferably, it can be encapsulated by the polymer. In this second case, the peptide that is encapsulated may be either a solid or in solution. It is preferred for the peptide to be a solid.

This formulation is useful in the treatment of human pathologies, including disorders of bone growth (including age-related osteoporosis and osteoporosis associated with post-menopausal hormone status, primary and secondary hyperparathyroidism, disuse osteoporosis, diabetes-related osteoporosis, and glucocorticoid-related

osteoporosis) and prostate growth (including benign prostatic hyperplasia and prostate cancer).

In a second aspect, the invention as disclosed herein comprises a method for the treatment of an individual suffering from a disorder of bone or prostate growth, or considered to be at risk of so suffering. This method of treatment comprises the administration to said individual of a therapeutically effective amount of a formulation containing, as an active principal, a peptide according to the sequence

pyroGlu-His-Trp-Ser-**Xaa**^{1}-Gly-**Xaa**^{2}-**Xaa**^{3}-Pro-Gly-NH_2 (7)

or a pharmaceutically acceptable salt thereof, wherein **Xaa**^{1}, **Xaa**^{2} and **Xaa**^{3} are as defined above, and as a second component, a pharmaceutically acceptable biodegradable polymer, which formulation releases the peptide into the systemic circulation as the polymer is eroded. The method of treatment may comprise a single administration of the formulation, but is more likely to comprise a course of repeated administrations. The frequency of the administrations may be from once per day to once per month. The amount of active peptide in each dose will depend on the dosing schedule and the route of administration. Generally, it will be between one milligram (1mg) and one gram (1g). The supervising physician will determine the precise dose depending on the parameters generally considered in the art to be relevant. The formulation is administered by intramuscular or subcutaneous injection.

The peptides that comprise the active agents of the compositions of the present invention can be prepared by the methods generally known in the art. For example, the peptides may be prepared by solid-phase synthesis. This involves the sequential addition of amino acid residues to a resin-bound intermediate according to the following strategy.

1. Formation of resin-bound first intermediate

   PG-Aaa-OH + FG-Res – PG-Aaa-L-Res

   Aaa = amino acid

   PG = protecting group

   FG = functional group

   Res = polymeric resin

L = linker group (-O- or -NH-)

2. Deprotection

PG-Aaa-L-Res - H-Aaa-L-Res

3. Chain extension

PG-Bbb-OH + H-Aaa-L-Res ~ PG-Bbb-Aaa-L-Res

4. Repeat steps 2 and 3 as necessary

PG-Bbb-Aaa-L-Res - - - PG-Nnn-...-Bbb-Aaa-L-Res

5. Cleave/deprotect

PG-Nnn-...-Bbb-Aaa-L-Res - H-Nnn-...-Bbb-Aaa-OH (or $-NH_2$)

In step one, a protected amino acid is reacted with a functionalised resin. The protecting group (PG) is most commonly *tert*-butyloxycarbonyl (Boc) or 9-fluorenylmethyloxycarbonyl (Fmoc). The functional group on the resin (FG) is commonly a chloroalkyl group, a hydroxyl group or an amine group. When FG is a chloroalkyl or hydroxyl group, the linker group (L) is an oxygen atom (-O-). When FG is an amine group, L is -NH-.

In step two, the protecting group (PG) is removed from the α-amino group. When PG is Boc, this can be accomplished by treating the resin with acids such as trifluoroacetic acid or hydrogen chloride in dichloromethane. When PG is Fmoc, the deprotection can be accomplished by treating the resin with bases such as piperidine.

In step three, the peptide chain is extended by one amino acid residue. A protected amino acid is coupled to the amine group liberated in step two. Many reagents are known in the art for achieving this conversion. One combination is dicyclohexylcarbodiimide (DCC) and hydroxybenzotriazole (HOBt). Generally, a base will also be necessary. Suitable bases include triethylamine and N,N-diisopropylethylamine. The solvent will generally be dichloromethane, dimethylformamide, or a mixture of these.

If the side chains of the amino acids (Aaa - Nnn) contain reactive groups (for example amino groups, carboxylic acid groups, hydroxyl groups) then these will need protecting. The protecting groups chosen for the side chains are generally those that are stable under the conditions required to remove the protecting group (PG) from the α-amino group. If PG is Fmoc, then the side chain protecting groups can conveniently be based on tert-butyl chemistry. On the other hand, if PG is Boc, then the side chain protecting groups can be based on fluorenylmethyl chemistry. Other protecting groups known in the art can also be used.

In step four, the deprotection/chain extension cycle is repeated until the desired peptide sequence has been constructed.

In step five, the completed peptide is liberated from the resin. Protecting groups are removed from the side chains either before or after the cleavage. When L is -NH-, the peptide liberated is in the form of the C-terminal amide. When L is -O-, the peptide liberated is often the C-terminal free acid and a second step is required to form the C-terminal amide.

The peptides may also be prepared by solution-phase synthesis, and this may be more convenient when large quantities of material are needed.

The polymers required for the formulation are generally well known in the art. As stated previously, the formulation may take the form of a simple dispersion of the peptide in a matrix of the polymer, or the peptide may be microencapsulated with the polymer. Dispersions can be prepared by mixing the peptide (as a solid) and the polymer to homogeneity, then compressing the mixture to form a solid mass. It may be necessary to add a binding agent to the mixture in order to achieve a suitably cohesive composition. The mass can then be ground up to give particles suitable for suspension in a biologically compatible liquid (such as water or isotonic saline) and injection.

Microencapsulated formulations can be prepared either from the solid peptide (as a powder) or from a solution, and particularly an aqueous solution, of the peptide. The polymer is first dissolved in a suitable organic solvent. The peptide is then added to this solution and the mixture is vigorously stirred to disperse the peptide in the organic phase. A second organic solvent is then added. This second solvent is

chosen to reduce the solubility of the polymer in the organic phase. The polymer comes out of solution to form a coating around the particles of solid peptide (or around the droplets of dispersed aqueous solution). The resultant microcapsules are then hardened by washing to remove traces of the organic solvents. They are then ready to be suspended in an appropriate liquid for administration.

The above general description is further elaborated below in a number of examples. These are intended to illustrate certain aspects of the invention. They are not intended to be limiting in any way.

EXAMPLES

Example 1 - Synthesis of GnRH-II

1A. Preparation of resin-bound protected peptide.

pyroGlu-His(Bom)-Trp(CHO)-Ser(Bzl)-His(Bom)-Gly-Trp(CHO)-Tyr(Bzl)-Pro-Gly-Ores

This peptide was prepared using standard solid-phase methods starting from Boc-Gly-esterified Merrifield resin (60 g, 1 mmol/g). The synthesis was performed in a manual synthesizer, with a total solvent and reagent volume of 300 mL for each operation. The standard deprotection/wash/coupling protocol is summarised in Table 1.

Table 1

Step	Reagent	Time (min)	Number of Operations
Deprotection of Boc	HCl/DCM*	60	1
Washing	DCM	2 - 4	3
Neutralisation	10% DIPEA/DCM	4	2
Washing	DCM	2 - 4	1
Coupling	Activated ester	60 - 120**	1 - 2
Washing	DCM	2 - 4	3

* Gaseous hydrogen chloride was bubbled through a suspension of the resin in DCM

** Completeness of reaction was determined by a negative ninhydrin test

Benzotriazolyl esters were used as the activated esters throughout the synthesis. These were prepared from the corresponding protected amino acids by reaction with 1-hydroxybenzotriazole (1 eq.) and dicyclohexylcarbodiimide (1 eq.). The quantities used (in relation to the resin substitution capacity) are listed in Table 2.

Table 2

Cycle no.	Amino acid derivative	Molar excess
1	Boc-Pro-OH	1. 8
2	Boc-Tyr(Bzl)-OH	1. 8
3	Boc-Trp(CHO)-OH	1. 8
4	Boc-Gly-OH	1. 8
5	Boc-His(Bom)-OH	1. 8
6	Boc-ser(Bzl)-OH	2.0
7	Boc-Trp(CHO)-OH	2.0
8	Boc-His(Bom)-OH	2.0
9	pyroGlu-OH	2.0

Following the final coupling. the resin was washed with dichloromethane (3 × 3 L) and dried under reduced pressure at +40°C to constant weight.

Amino acid analysis: Consistent with proposed sequence

1B. Cleavage and deprotection

pyroGlu-His-Trp-Ser-His-Gly-Trp-Tyr-Pro-Gly-NH_2 (6)

The peptidoresin prepared in Example 1A was placed in a linen bag in a pressure vessel. The vessel was then charged with gaseous ammonia to a final pressure of 4 atm. After 72h the excess ammonia was vented and the resin was extracted with acetic acid (3×100mL) and ethanol (3×100mL). The combined extracts were degassed with nitrogen, 10% palladium-on-carbon was added, and the mixture was stirred under an atmosphere of hydrogen. When the reaction was complete (as judged by HPLC), the mixture was filtered and the filtrate was evaporated. The residue was purified by reverse-phase HPLC to give the title compound.

Example 2- Microencapsulation of peptide

Copoly(D,L-lactic acid, glycolic acid) with a lactic acid/glycolic acid ratio of 50/50 is used. To a solution of this polymer (3.7g) in dichloromethane (100mL) in a reaction vessel equipped with a stirrer is added GnRH-II acetate (0.15g, prepared by dissolving the peptide of example 1 in acetic acid and lyophilising the resultant solution). The mixture is stirred at 500revolutions/minute, then silicone oil (Dow Corning 360 Medical Fluid®, 45g) is added over 10 minutes. The mixture is then introduced as a thin jet into caprylic-capric acid-triglyceride (Miglyol® 812, 3.3L) with continuous stirring at 1000revolutions/minute. When addition is complete, stirring is continued for 1 hour, then the microcapsules are collected by filtration, washed twice with isopropanol, and finally dried.

Example 3 - Analysis of the effects of GnRH-II and analogues on Osteogenic cell populations *in vitro*.

(a) Human osteoblasts were isolated from cancerous bone from orthopaedic surgery (Nilsson *et al.*, 1995) according to standard procedures known in the art. The bone

explants were minced into small bone chips and then washed extensively in Dulbecco's modified Eagle's medium (DMEM)/F12 (1:1 Gibco, Paisley, U.K). These osteoblast like cells, Murine osteoblastic MC3T3-E1 cells and human clonal osteosarcoma cell lines MG-63 (non-mineralising) and SaOS-2 (mineralising osteosarcoma) were cultured in DMEM:F12, 1:1 with the addition of 10% fetal calf serum (FCS, Gibco), fungizone (500mg/l), gentamycin sulphate (50mg/l), L-glutamine (2mM) and l-ascorbic acid (100mg/l) in a humidified CO_2 chamber at 37°C.

(b) Human bone marrow stromal cells were isolated from bone fragments rinsed in phosphate-buffered saline. Bone marrow cells were collected and spun through a column of Ficoll Hypaque (Kimble *et al* J. Clin. Invest. 93 1959-1967, 1994) Cells at the interface were pelleted, counted and seeded into 75cm^2 flasks. The cells were incubated in a humidified CO_2 chamber at 37°C and the medium changed weekly. At confluence, the cells were harvested using trypsin EDTA and re-seeded in α-minimum essential medium (α-MEM) supplemented with 10% fetal calf serum (FCS, Gibco), penicillin (100U/ml), streptomycin (100mg/ml), fungizone and L-glutamine (2mM).

(c) All cells were serum-starved for 48h before addition of GnRH-I and GnRH-II. Cells were placed in DMEM without phenol red (in order to avoid oestrogen-like effects of phenol red) containing 10% charcoal-stripped serum for 48 hours in 12 well plates. Dose dependent effects of GnRH-I and GnRH-II and analogues of the peptides were studied following the addition of peptides at final concentrations ranging from 10^{-9} to 10^{-6}M. 1mM dibutyryl cAMP was used as a control. The cells were incubated for 24, 48 and 96h with the peptide being replaced every 24 hours.

(d) TO assess the effects of the peptides on cell proliferation, [^{3}H]thymidine was added at 1mCi/ml for an additional 24hours and [^{3}H]thymidine incorporation was determined. Radioisotope incorporation was determined using a scintillation counter and the results were calculated as cpm/mg of total protein.

(e) Expression of osteoblastic differentiation markers was also determined (Tintut Y *et al*,. J Biol Chem 273 7547-53, 1998). Total RNA was isolated at several stages

before treatment, at 24, 48, 72 and 96 hours after addition of peptides. Type I procollagen, osteopontin and 28S RNA (used as an internal control) expression was determined by Northern blot analyses. Alkaline phosphatase, matrix GLA protein, osteoclastin and GAPDH (as an internal control) were determined by RT-PCR with specific primers designed for each gene.

The peptides of the invention caused significant effects at concentrations below 100μM.

Example 4 - Analysis of the effects of GnRH-II and analogues on Osteoclast populations *in vitro*.

(a) Human clonal cell lines of osteoclast precursors (FLG 29.1) were used as an *in vitro* model of osteoclast differentiation (Gattei V *et al.*, Cell Growth Differ 7 753-63, 1996). In addition, co-cultures of FLG 29.1 and osteoblastic cells (Saos-2) were evaluated for migratory, adhesive, cytochemical, morphological, and biochemical changes. Dose dependent effects of GnRH-I and GnRH-II and analogues of the peptides were studied following addition at final concentrations ranging from 10^{-9} to 10^{-6}M to FLG 29.1 cultures and to co-cultures. Parathyroid hormone was added as a control. Potentiation (or inhibition) of the differentiation of the preosteoclasts (fusion into large multinucleated elements) and a number of other factors were measured (Orlandini *et al*,. Cell Tissue Res. 281 33-42, 1995). These included:

1. Positive staining for tartrate-resistant acid phosphatase in FLG 29.1 cells
2. A decrease of the alkaline phosphatase activity expressed by Saos-2 cells
3. The appearance of typical ultrastructural features of mature osteoclasts in FLG 29.1 cells
4. The release into the culture medium of granulocyte-macrophage colony stimulating factor.
5. To assess the effects the peptides on cell proliferation, [^{3}H]thymidine was added at 1mCi/ml for an additional 24hours and [^{3}H]thymidine incorporation was determined as described above.

(b) Bone marrow cells removed from human bone fragments were cultured in the presence of 10nM 1,25-$(OH)_2$ vitamin D_3 for seven days to generate multinucleated osteoclasts using standard techniques known in the art (Takahashi *et al.*, Endocrinol 122 1473-1482, 1988). The culture medium (α-MEM) was removed and replaced by a fresh phenol red free medium supplemented with antibiotics and 10% charcoal-stripped heat-inactivated FCS containing GnRH-I, GnRH-II or

analogues, and the cultures were maintained for a further 24 hours. Floating cells were harvested and osteoclasts stained for tartrate-resistant acid phosphatase (TRAP) expression, a marker of osteoclast differentiation (Hughes *et al.*, Nat. Med. 2 1132-1135, 1996)

1. Cells were incubated in 0.2M acetate buffer, pH 4.7-5.0, containing tartaric acid and 2% naphthol AS-BI phosphate (dissolved at 20mg/ml in ethylene glycol monomethyl ether) for 15min at 37°C. The cells were then transferred to a second solution consisting of the same buffer and concentration of tartaric acid with 0.1% pararosanoiline chloride (hexazotised by mixing with an equal volume of 4% sodium nitrite for 5min at room temperature) for 10min at 37°C. This treatment causes a red cytoplasmic stain in cells expressing TRAP. Harris' hematoxylin was used as a nuclear counterstain.
2. Apoptotic multinulceated osteoclasts were identified by strong expression of TRAP, larger size than accompanying viable TRAP-positive cells. Confirmation of apoptosis was carried out using acridine orange stain. Viable osteoclasts were counted after fixation in 95% ethanol and TRAP hematoxylin staining, and apoptotic osteoclasts were expressed as a percentage of the total number of multinucleated osteoclasts (viable and apoptotic) in each culture well.

The peptides of the invention caused significant effects at concentrations below 100μM.

Example 5- Expression analysis of GnRH mRNA in osteogenic and osteoclast cell populations

Total RNA was extracted from cells cultured as described above:

1. osteoblast like cells, isolated from cancerous bone
2. murine osteoblastic MC3T3-E1 cells
3. MG-63 (non-mineralising)
4. SaOS-2 (mineralising osteosarcoma)
5. human bone marrow stromal cells
6. human FLG 29.1 osteoclast precursor cells
7. multinucleated osteoclasts generated from bone marrow

Expression of GnRH-I and GnRH-II was determined by RT-PCR using PCR primers outlined in SEQ I.D. No 1-4. The integrity of the cDNA generated was determined by assessing the relative level of actin amplification.

Example 6 - Effect of GnRH-II on bone mineral density in the ovariectomised rat

(a) Female adult (8 weeks old, 200-215g) Sprague Dawley rats were bilaterally ovariectomised (OVX). Animals were kept for 4 weeks post-delivery before commencing treatment. Purina rat chow (1.00% calcium. 0.61% phosphorous) and water were provided ad libitum. Each study consisted of 6 weight-matched groups (n = 8/group).

(b) Treatment started 4 weeks post-OVX. After 4 weeks, a baseline control OVX group was sacrificed (Group A). The remaining groups were injected once a day with vehicle (Group B), 1μg/kg body weight (Group C), 10μg/kg body weight (Group D), 100μg/kg body weight (Group E) of GnRH-II, and 80μg/kg body weight (Group F) of hPTH(1-34).

(c) All rats were weighed every fourth day and dosages adjusted for 50g increase in mean group weight. Rats were given alternate subcutaneous injections of calcein (30mg/kg) or tetracyclin (30mg/kg) in 2% sodium bicarbonate-saline, respectively to label mineralization surfaces on days 10, 19 and 26, following treatment with drug. Bone mineral density was assessed by dual energy x-ray absorptometry-DEXA). On day 28 serum calcium levels were determined by colorimetric assay using a commercial kit.

(d) Success of OVX was confirmed at necropsy by failure to detect ovarian tissue and by observation of marked atrophy of the uterine horns. Both legs were disarticulated at the hip. The left tibia and femur were cleaned of excess muscle and soft tissue and placed in 70% ethanol. The anterior eminence of the right tibia metaphysis was shaved with a razor blade, barely exposing bone marrow. Both right femur and tibia were then placed in 10% phosphate-buffered formalin for 24h and transferred to 70% ethanol.

Ovariectomised animals treated daily with 10 and 100µg/kg of GnRH-II and 80µg/kg PTH for 28days have pronounced hypercalcemia. Results are shown in Figure 1.

Example 7 - Cellular localisation of GnRH-II in paraffin sections of normal rat bone and human bon .

(a) Frozen and/or paraffin-embedded human and rat bone sections were fixed for 3-36h depending on size (3-5h at room temperature, then approx 24h at 4°C) and then soaked in 0.1M Tris + 5 % EDTA (12.11g + 50g EDTA) pH 7.3 until decalicified.

(b) Sections were then processed for antibody staining (rabbit polyclonal anti-GnRH-II antibody) using standard techniques.

Staining for GnRH-II was observed in platelets, megakaryocytes at the growth plate (especially proliferating chondrocytes). Some staining was also seen in the bone-forming cells particularly in active osetoblasts as well as new osteoid.

Example 1 demonstrates the preparation of the peptides of the invention, which can then be formulated as illustrated in Example 2. Examples 3 to 7 demonstrate the biological activity of the peptides of interest. The scope of the invention is not intended to be limited in any way by these Examples. In particular, it will be realised that variety of controlled release formulations of these peptides can be prepared by varying the polymer and/or the physical nature of the combination of the peptide and polymer. However, these variations give formulations with equivalent biological properties, and are intended to be within the scope of the invention as defined in the following Claims.

SEQ I.D. Nos. 1 to 4 referred to in Example 5 are as follows :

CTG	CAG	CTG	CCT	GAA	GGA	C	(1)
GGG	CGG	GGC	GGG	GCT	CTC	G	(2)
ATT	CTA	CTG	ACT	TGG	TGC	GTG	(3)
GGA	ATA	TGT	GCA	ACT	TGG	TGT	(4)

CLAIMS

1. A pharmaceutical formulation for the controlled release of a therapeutic peptide or a salt thereof, which peptide has the sequence

pyroGlu-His-Trp-Ser-**Xaa**^{1}-Gly-**Xaa**^{2}-**Xaa**^{3}-Pro-Gly-NH_2

wherein **Xaa**^{1} is His or Tyr,
Xaa^2 is Trp or Leu, and
Xaa^3 is Tyr or Arg,

provided that when **Xaa**^{1} is Tyr and **Xaa**^{2} is Leu, then **Xaa**^{3} is not Arg,

and which formulation further comprises a pharmaceutically acceptable biodegradable polymer.

2. The pharmaceutical composition according to Claim 1, wherein the peptide is

pyroGlu-His-Trp-Ser-His-Gly-Trp-Tyr-Pro-Gly-NH_2

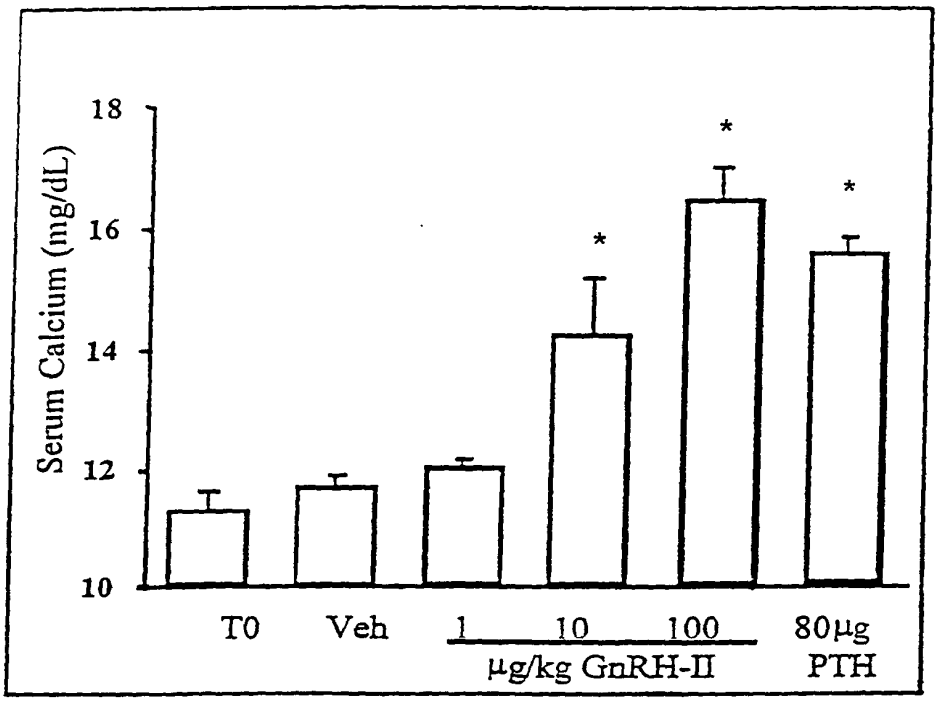
3. The formulation according to Claim 1, wherein the polymer is a polymer of a hydroxy derivative of a carboxylic acid, or a copolymer of such derivatives.

4. The formulation according to Claim 3, wherein the polymer is a polymer of glycolic acid, a polymer of lactic acid, or a copolymer of lactic and glycolic acids.

5. The formulation according to Claim 1 wherein the peptide is microencapsulated by the polymer.

6. A method for the treatment of a human medical condition, which method comprises the administration to an individual in need of such treatment of a therapeutically effective amount of a controlled release formulation of a peptide according to any of the preceding Claims.

Effects of GnRH-II at various doses on serum levels of calcium



SEQUENCE LISTING

(1) GENERAL INFORMATION:

(i) APPLICANT:
(A) NAME: FERRING BV
(B) STREET: MARSSTRAAT 9, PO BOX 3129
(C) CITY: HOOFDDORP
(D) STATE: NONE
(E) COUNTRY: THE NETHERLANDS
(F) POSTAL CODE (ZIP): 2130 KC

(ii) TITLE OF INVENTION: CONTROLLED RELEASE FORMULATION

(iii) NUMBER OF SEQUENCES: 7

(iv) COMPUTER READABLE FORM:
(A) MEDIUM TYPE: Floppy disk
(B) COMPUTER: IBM PC compatible
(C) OPERATING SYSTEM: PC-DOS/MS-DOS
(D) SOFTWARE: PatentIn Release #1.0, Version #1.30 (EPO)

(2) INFORMATION FOR SEQ ID NO: 1:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 19 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 1:

CTGCAGCTGC CTGAAGGAG 19

(2) INFORMATION FOR SEQ ID NO: 2:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 19 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 2:

GGGCGGGGCG GGGCTCTCG 19

(2) INFORMATION FOR SEQ ID NO: 3:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 21 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 3:

ATTCTACTGA CTTGGTGCGT G 21

(2) INFORMATION FOR SEQ ID NO: 4:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 21 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 4:

GGAATATGTG CAACTTGGTG T 21

(2) INFORMATION FOR SEQ ID NO: 5:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 10 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(ix) FEATURE:
(A) NAME/KEY: Modified-site
(B) LOCATION:1
(D) OTHER INFORMATION:/product= "Glu in first position is pyroGLU"

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 5:

Glu His Trp Ser Tyr Gly Leu Arg Pro Gly
1 5 10

(2) INFORMATION FOR SEQ ID NO: 6:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 10 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(ix) FEATURE:
(A) NAME/KEY: Modified-site
(B) LOCATION:1
(D) OTHER INFORMATION:/product= "Glu in first position is pyroGlu"

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 6:

Glu His Trp Ser His Gly Trp Tyr Pro Gly
1 5 10

(2) INFORMATION FOR SEQ ID NO: 7:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 10 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(ix) FEATURE:
(A) NAME/KEY: Modified-site
(B) LOCATION:1
(D) OTHER INFORMATION:/product= "Glu is pyroGlu"

(ix) FEATURE:
(A) NAME/KEY: Modified-site
(B) LOCATION:5
(D) OTHER INFORMATION:/product= "Xaa is His or Tyr"

(ix) FEATURE:
(A) NAME/KEY: Modified-site
(B) LOCATION:7
(D) OTHER INFORMATION:/product= "Xaa is Trp or Leu"

(ix) FEATURE:
(A) NAME/KEY: Modified-site
(B) LOCATION:8
(D) OTHER INFORMATION:/product= "Xaa is Tyr or Arg"

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 7:

Glu His Trp Ser Xaa Gly Xaa Xaa Pro Gly
1 5 10